IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	'COMPOUNDS'
HUGH CAIRNS et al	:	Group Art Unit 122
Serial No 344,982	:	David B Springer, Examiner
Filed 2 January 1982	:	

DECLARATION

I, RAYMOND WILLIAM KEOGH, declare and say as follows:

I am a subject of the Queen of Great Britain and reside at 24 Harewood Close, Langham, Rutland, England.

I have obtained the degree of Bachelor of Science (Medical Biochemistry) (1966) and Doctor of Philosophy (Experimental Pathology) (1970) from the University of Birmingham, England. I am also a Member of the British Society for Immunology.

Since 1970 I have worked in the Research and Development Laboratories of the Pharmaceutical Division of Fisons plc and currently hold the post of Head of Immunology in those Laboratories.

The following test has been carried out under my supervision.

Sprague Dawley rats (Charles River/CD), having a body weight of 200-250g, were injected intramuscularly in the hind leg with 1mg chicken egg albumen (OA) in 0.2ml isotonic saline. This was followed immediately by intrapentoneal injection of 0.5ml

B.pertussis vaccine (Lister) as adjuvant, and the rats were bled by heart puncture two weeks later.

The blood cells were removed by centrifugation, and the sera collected to provide a pool of serum containing IgE antibody to OA. The serum was titrated using the test described below, to determine the amount required to give a skin weal 2cm in diameter. This response was obtained using serum diluted 1 in 4 with isotonic saline. This diluted solution is called serum A.

Charles River/CD female rats weighing approximately 100g were sensitised by intradermal injection of 0.1ml serum A into the right flank. Sensitivity was allowed to develop for 24 hours, and the rats were then injected intravenously with 1ml of a mixture of 0.25ml OA (10mg/ml in isotonic saline), 0.25ml Evans blue dye, and the solution of the compound under test (0.5ml varying percentages of compound). For each percentage level of test compound, five rats were injected. Five rats were used as controls in each test. The doses of each compound under test were selected so as to give a range of inhibition values.

Twenty minutes after injection of the antigen (OA) mixture, the rats were killed and the skins reversed to show the reaction more clearly. The intensity of the anaphylactic reaction was assessed by comparing the size of the characteristic blue weal produced by spread of the Evans blue dye from the sensitisation site with the size of weal in the control animals. The size of weal was recorded as the mean of two opposing diameters, and the percentage inhibition for each dose level calculated as:-

$$\% \text{ Inhibition} = \frac{(\text{Control group weal} - \text{Treated group weal}) \times 100}{\text{Control group weal}}$$

The percentage inhibitions for the various dose levels were plotted graphically for each compound. From these graphs the dosage required to achieve a 50% inhibition of the anaphylactic reaction (ID_{50}) was determined. The results are shown in the following table.

Table

Compound	ID_{50} mg/kg
Compound of Serial No 344,982	
Disodium 4,6-dioxo-10-propyl-4H,6H-pyrano-[3,2-g]quinoline-2,8-dicarboxylate	0.02
Compound of Japanese Patent Application 073427	
Sodium 6,9-dihydro-6-methyl-9-oxo-2H-pyrano (2,3-g) quinoline-8-carboxylic acid	>10

The test method set out above is recognised as giving a reliable measure of a compound's ability, when administered i.v., to inhibit an antigen-antibody reaction, as is evidenced by the following publications all of which use simultaneous administration of drug, antigen and dye in the PCA test:-

D HOLLAND, et al J Med Chem (1976) 19, 1225 - 1228.
Cinnoline-3-propionic acids. A new series of orally active anti-allergic substances. (See page 1228).

J R PFISTER, et al, J Med Chem (1978) 21, 669 - 672.
Synthesis and anti-allergic activity of mono and disubstituted xanthone-2-carboxylic acids. (See page 672).

J B WRIGHT et al, J Med Chem (1978) 21, 930 - 935.
N.N[1] (Phenylene) dioxamic acids and their esters as anti-allergy agents. (See page 935).

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge tha wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

this 24th day of January 1984

Declarant R. K. [illegible]

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